Transiently Increased Variation Between a Point-of-Care and Laboratory INR Method After a Long Period of Correlation

A Case Study Demonstrating the Need for Ongoing Correlation of POC With the Central Laboratory

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ABSTRACT

Objectives: To perform long-term comparison between laboratory Stago and Point-of-Care (POC) i-STAT methods for determining the international normalized ratio (INR).

Methods: This was a multicenter method comparison of patient INR results and factors related to performance variance.

Results: For 5 years, the assays demonstrated close patient correlation within and above the 3.5 INR therapeutic range cutoff (bias, 0.23 INR units). Patient results above 3.5 INR were bimodal, with 60% demonstrating an i-STAT INR bias of less than 0.5. Several patient conditions were associated with the presence of a higher i-STAT INR bias of less than 0.5. In year 6, a broader range i-STAT bias developed, increasing to 0.73 INR units. The increased bias persisted for 3 years, then returned to initial levels following i-STAT adjustments. The substantial increase in i-STAT bias after a long period of stability was partly corrected by renewed correlation to the international reference preparation. Additional assay drift is discussed in relation to thromboplastin reagents and other testing variables.

Conclusions: This study emphasizes the need for continual laboratory correlation with POC devices and caution in using published comparisons.

Point-of-care (POC) and self-monitoring of warfarin are widely used. These methods are convenient for both patient and provider relative to standard phlebotomy and central laboratory testing of venous blood. Improved precision and accuracy of prothrombin time (PT) monitoring and international normalized ratio (INR) calculations with POC devices have reduced the incidence of bleeding and thrombosis in patients treated with warfarin.1 The INR is determined by calibrating laboratory-based commercial thromboplastin reagents to the international reference preparation (IRP) and using the slope of the plotted log PTs to establish an international sensitivity index (ISI).

Despite assay and instrument improvements, a number of variables remain that complicate monitoring of warfarin therapy, independent of testing precision or INR calculations. The variables include different thromboplastin sources associated with the IRP, continued INR variation among detection systems and reference materials, variation in diet and warfarin metabolism among patients, and the effects of patient condition on the PT/INR assay. One systematic review with metaregression analysis of 67 studies found that patients receiving anticoagulation therapy fell outside the therapeutic range 36.4% of the time.2 Many pharmaceutical compounds and foods with higher concentrations of vitamin K significantly influence the INR in patients treated with warfarin.3 Because of genetic variability, some patients are highly sensitive to warfarin and require lower doses to maintain a therapeutic INR.3 In addition to patient variation within a testing system, assay disagreements between central laboratory systems and POC systems remain common, resulting in difficulties in assessing patients and managing warfarin therapy in more than 1 assay system. More than 30 instruments and more than...
100 instrument-reagent combinations are used in the central laboratory alongside a lesser number of POC INR assays (in the United States, 16 instruments are available from 8 companies).\textsuperscript{9,10} It is helpful to understand the relationship between the venous sample tested in the laboratory and the fingerstick POC test when making dosing decisions. When disagreements exist, the expected query from the clinician is, “Which one is right?” It is therefore necessary for the laboratory to understand what factors may lead to disagreement between the central laboratory and a POC test.

Stago (Diagnostica-Stago, Parsippany, NJ) and i-STAT (Abbott, Abbott Park, IL) are common central laboratory and POC systems, respectively, in US hospitals. To our knowledge, 3 reports have compared i-STAT POC INR analyzers to central laboratory tests.\textsuperscript{11-13} Karon et al\textsuperscript{13} found that more than 90% of the results on the i-STAT were within 0.4 INR units of a central laboratory Trinity MDA 180 analyzer (Fisher Scientific, Pittsburgh, PA). Boellesen et al\textsuperscript{11} demonstrated that the INR measured with the i-STAT portable analyzer was precise and compared well with a central laboratory Dade BCS analyzer (Siemens, Malvern, PA) with a bias of less than 0.2 for INR values at or below 3.5. A more recent study compared INR values between the Stago central laboratory analyzer and i-STAT and CoaguChek XS Plus (Roche, Indianapolis, IN) POC analyzers.\textsuperscript{12} This 3-month study of 52 patients reported a greater i-STAT bias of 0.51 units with about one-third of the i-STAT values falling above 3.5 INR. The earlier studies reported a closer correlation between fingerstick i-STAT and venous plasma central laboratory INR values than the more recent study. Differences in assay bias may result because comparisons use different central laboratory assays or because assays change over time. These studies were limited to approximately 50 patients tested at a single institution, with most compared values falling within the therapeutic range. Comparisons were typically done over short periods, usually several months, and did not relate patient condition or sample type to variance.

The current study incorporates data from several institutions within a Veterans Administration (VA) Hospital Network in the United States evaluating the i-STAT INR, using human recombinant thromboplastin with a central laboratory Stago INR that uses Neoplastine CL Plus rabbit thromboplastin (Diagnostica-Stago). The rationale for these studies was severalfold: (1) to examine the correlation of a POC and laboratory system using a large number of patients from multiple laboratories in a hospital network; (2) to compare the stability of assay bias over an extended period with changes in manufacturer lots of thromboplastin reagents for both assays; (3) to compare laboratory INR results on plasma samples to a POC device using either whole blood from capillary fingerstick or fresh venous whole blood drawn in a plain plastic Vacutainer; (4) to compare the assays for patients with INR values within and outside the therapeutic range; and (5) to investigate the effect of patient conditions on assay results and assay bias.

The average assay bias for this large population of patients receiving warfarin was unexpectedly small and stable over a period of 5 years, with numerous changes in thromboplastin lots. Over this period, a small positive INR bias was seen in the i-STAT compared with the Stago, and it was slightly greater for capillary whole blood samples than for venous whole blood samples. Among the INR values above 3.5, the early i-STAT–positive bias was significant for 42% of the patient values. Significant patient INR bias was, in some instances, associated with the effect of particular medical conditions combined with effects of sample type and assay type. After 5 years of low and stable bias, a large increase in i-STAT–positive bias developed and persisted for nearly 3 years. This reflected a change in reagent performance without changes in standardization or ISI designation from manufacturers. In year 9 (2012), the i-STAT bias was corrected by re correlating to a laboratory plasma assay with traceable standardization to the most recent (2009) international reference plasma. Laboratories are typically unaware of all aspects of manufacturing variance and therefore must be persistent in performing correlation studies between POC and laboratory INR tests.

Materials and Methods

Testing Systems

The i-STAT PT/INR test uses human recombinant thromboplastin and results in thrombin cleavage of a synthetic substrate, the product of which is read amperometrically. This test may be performed on fresh unanticoagulated venous blood or fingerstick capillary whole blood specimens. Correlation studies with the Stago PT/INR test were performed using both sample types. i-STAT sample volume must be between 20 and 45 μL, the hematocrit should lie between 24% and 54%, and the fibrinogen must be between 70 and 541 mg/dL (method not specified).

The Stago Compact test uses Neoplastine CL rabbit thromboplastin and results in thrombin generation, which produces a fibrin clot detected mechanically by monitoring movement of a metal ball oscillating via electromagnetic pulses between different sides of an arc on the bottom of the cuvette. The end point is reached when the movement of the ball is inhibited by the fibrin clot and ceases to move.

Patients, Samples, and Studies

The patients studied attended warfarin clinics at 4 network hospitals managed by nurses and Doctors of Pharmacy. Patient consents were obtained to collect fingerstick and
venous specimens when the i-STAT assay initiation studies were performed. Venous specimens were either collected as citrated plasma tested on the laboratory instrument (Stago) or fresh whole blood. They were collected from 114 patients for method correlation studies for implementation of the i-STAT test in the clinics. Fingerstick whole blood specimens were also assayed on the i-STAT for new method correlations and all subsequent studies. Following new method studies, only fingerstick i-STAT and plasma Stago correlation studies were performed for quality assurance and for further investigation of bias changes. The fingerstick i-STAT comparison to the Stago method included 169 randomly selected patients with a broad range of INR results. In this group, a subgroup of patients with i-STAT INR levels higher than 3.5 were specifically selected for separate analysis. Eight patients with INR values above the therapeutic range demonstrated higher-than-normal i-STAT bias, and multiple paired i-STAT and Stago results over time in these patients were further studied. The clinical records of these patients were reviewed retrospectively for possible contributory causes of the discordant results. These included patient conditions associated with reduced coagulation factors or with altered capillary perfusion that may contribute to deviation from expected assay bias between the 2 assays.

An additional study was then conducted to evaluate a new and substantial increase in the proportional bias of i-STAT INR levels compared with the Stago values. This increase occurred after 5 years of a relatively stable bias. In this study, the fingerstick i-STAT INR was compared with the Stago central laboratory INR in 196 randomly selected patients from 2 hospitals. A final study was conducted following a manufacturer correction of i-STAT bias. This investigation compared i-STAT fingerstick results to Stago plasma results in 103 randomly selected patients. In addition to the average bias of the larger comparison studies, bias over the first 5 years was also determined from individual year correlation quality assurance data to illustrate the consistency in early correlation. In some instances, data were separated into groups based on INR results below and above 3.5 INR and based on patient differences between the 2 methods that varied by less than or greater than 0.5 INR units. The 0.5 INR cutoff was chosen as meaningful. It was used to assess correlation and bias changes and not used clinically to evaluate method agreement for warfarin dosing.

Data for initiation studies were collected from 4 hospitals, and data for 6-month correlation studies were collected from 3 hospitals in a hospital network standardized for equipment and reagents. Stago reagent lot numbers were standardized with lot changes occurring annually. All venous samples were collected in plastic tubes. Method correlations were performed in 2 ways: (1) by comparing the INR of fresh unanticoagulated venous blood on the i-STAT to the INR of plasma separated from sodium citrate (3.2%) anticoagulated blood after centrifugation at ×1,500g for 10 minutes to generate platelet-poor plasma, and (2) by comparing the INR of fingerstick capillary whole blood results on the i-STAT to the INR of sodium citrate (3.2%) anticoagulated plasma after centrifugation at ×1,500g for 10 minutes to generate platelet-poor plasma.

Thromboplastin Reagents

Data were collected over multiple years with changes in thromboplastin reagent lot numbers. Thromboplastin reagents for plasma-based tests are standardized to a World Health Organization (WHO) IRP. The slope of the log-log plot determines the ISI assigned to the thromboplastin reagent and used in the INR calculation. The Stago rabbit thromboplastin (Neoplastine Cl Plus) reagent lots were standardized within the hospital network, with annual changes showing slight variation in ISI values (year 1, ISI = 1.33; year 2, ISI = 1.26; year 3, ISI = 1.26; year 4, ISI = 1.32; year 5, ISI = 1.22; year 6, ISI = 1.28; year 7, ISI = 1.25; year 8, ISI = 1.25; year 9, ISI = 1.29). Annual mean normal PT (geometric mean) was determined with each reagent change and remained within the range of 12.8 to 13.5, typically 13 seconds.

The i-STAT human recombinant thromboplastin reagent lots are manufactured with a constant ISI of 1.05 and mean normal time of 12 seconds. Reagent lots changed approximately 4 to 5 times per year with no published change in ISI or mean normal PT.

Stago thromboplastin reagents were calibrated by the manufacturer using plasma samples compared with WHO IRPs for rabbit thromboplastins RBT/90 or RBT/05, the second- and third-generation rabbit WHO IRPs. Human recombinant thromboplastin IRPs available during this period were rTF/95 and the fourth-generation rTF/09. The exact laboratory-based method used to establish the i-STAT whole blood assay traceability to the WHO reference plasma is unknown. However, based on inquiries to the manufacturer, i-STAT reagents were not standardized to rTF/09 until 2012.

Statistics

Data were plotted, and nonregression statistics used Microsoft Excel software (Microsoft, Redmond, WA). Orthogonal regression was calculated and plotted via the Deming method using EP Evaluator (Rhodes). Bland-Altman analyses were calculated and plotted using Microsoft Excel.

Results

Long-Term Assay Correlation: Loss and Recovery of Close Correlation

When i-STAT testing was initiated across the network, INR test results were collected as both venous whole blood
and fingerstick capillary blood. Both i-STAT sample types were compared using Deming orthogonal regression analysis of results for plasma samples analyzed on Stago STA-Compact analyzers \textit{Figure 1A} and \textit{Figure 1B}. Compared with plasma results on the Stago analyzer, venous whole blood tested with the i-STAT had a small positive 0.1 average INR bias, whereas fingerstick capillary whole blood samples had an average INR bias of 0.23 INR over the first 5 years \textit{Figure 2A}, \textit{Figure 2B}, and \textit{Table 1}. The Deming correlation of i-STAT to Stago had a coefficient ($R^2$) of 0.97 for i-STAT tests performed on whole blood from tubes and 0.93 for those tested on capillary fingerstick samples (Figure 1, Table 1). Compared with the Stago analyzer, the i-STAT tests with fingerstick capillary blood were moderately increased compared with venous whole blood samples (Figure 1A, Figure 1B, Figure 2A, Figure 2B, and Table 1).

\textbf{Figure 1} Deming regression of paired i-STAT and Stago international normalized ratio (INR) values. i-STAT INR comparison studies with Stago were performed either by collecting fresh venous blood in a plain plastic tube (A) during initial correlation studies or by using capillary fingerstick blood during initial and ensuing correlation studies (B-D). Both i-STAT sample types were compared with venous plasma samples run on the Stago Compact and analyzed for correlation and linear fit using the Deming or orthogonal regression methods. 

- **A**, venous whole blood tested fresh from the tube on i-STAT vs venous plasma on the Stago; INR to less than 6; data from 4 sites (slope = 1.13; intercept = −0.213; $R = 0.97$); 
- **B**, fingerstick capillary blood on i-STAT vs plasma on Stago for first 5 years; data from 4 sites (slope = 1.08; intercept = −0.003; $R = 0.93$); 
- **C**, fingerstick capillary blood on i-STAT vs plasma on Stago after year 6 to assess the shift in i-STAT bias; data from 3 sites (slope = 1.48; intercept = −0.066; $R = 0.93$); 
- **D**, fingerstick capillary blood vs Stago correlation study performed to evaluate data following the correction in the i-STAT shift in year 9 (slope = 1.18; intercept = −0.45; $R = 0.92$).
Subsequent comparison studies used only fingerstick whole blood for i-STAT results, which is the sample that is used for routine patient testing. Capillary specimens may contribute to assay bias in patients with certain health conditions, as seen in subsequent data associated with individual patient variation.

The POC and laboratory methods were compared every 6 months or when increased discrepancy was suspected (annual bias presented in Table 2). Over the first 5 years of the study, the 2 methods demonstrated low overall bias (Table 1 and Table 2). As expected, beyond INR therapeutic limits of 3.0 and 3.5, there was less consistency. A surprising number of samples correlated well, whereas others were significantly higher than the Stago results (Figure 1B). Assay correlation dramatically changed 6 years after implementation of the i-STAT assay, when there was a notable increase in bias and slope compared with the Stago assay (Figure 1C) and Figure 2C (Tables 1 and 2). Providers noted that i-STAT results above an INR therapeutic limit of 3.0 and 3.5 were more common and more likely to have significantly higher values than those with the Stago (Figure 1C) and Figure 3. After 3 years of a significantly increased bias, the i-STAT results were adjusted by the manufacturer. The bias and slope in year 9 improved and were similar to levels...
Table I
Comparative Bias and Regression Statistics for Different Years Illustrating Changes in i-STAT and Stago Correlation

<table>
<thead>
<tr>
<th>INR Data Source</th>
<th>No.</th>
<th>Deming Regression/Slope</th>
<th>i-STAT-Stago (Average Bias ± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial 5 years: low bias</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>INR Range: i-STAT venous whole blood vs Stago</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0-6.2</td>
<td>114</td>
<td>0.97/1.13</td>
<td>0.10 ± 0.28</td>
</tr>
<tr>
<td>0-3.5</td>
<td>96</td>
<td>0.94/1.03</td>
<td>0.05 ± 0.21</td>
</tr>
<tr>
<td>&gt;3.5</td>
<td>18</td>
<td>0.91/1.08</td>
<td>0.40 ± 0.40</td>
</tr>
<tr>
<td>INR Range: i-STAT fingerstick vs Stago</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0-5.6</td>
<td>169</td>
<td>0.93/1.08</td>
<td>0.23 ± 0.40</td>
</tr>
<tr>
<td>0-3.5</td>
<td>119</td>
<td>0.92/0.88</td>
<td>0.14 ± 0.24</td>
</tr>
<tr>
<td>&gt;3.5</td>
<td>50</td>
<td>0.51/0.80</td>
<td>0.43 ± 0.59</td>
</tr>
<tr>
<td>&gt;3.5 with bias ≤0.5 relative to Stago</td>
<td>29</td>
<td>0.91/0.82</td>
<td>0.10 ± 0.25</td>
</tr>
<tr>
<td>&gt;3.5 with bias &gt;0.5 relative to Stago</td>
<td>21</td>
<td>0.73/1.22</td>
<td>1.01 ± 0.39</td>
</tr>
<tr>
<td>Combined multiple results of 8 high INR patients</td>
<td>65</td>
<td>0.81/1.45</td>
<td>1.36 ± 0.28</td>
</tr>
<tr>
<td>Years 6-8: increased bias</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>INR Range: i-STAT fingerstick vs Stago</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0-7.2</td>
<td>196</td>
<td>0.93/1.48</td>
<td>0.73 ± 0.59</td>
</tr>
<tr>
<td>0-3.5</td>
<td>90</td>
<td>0.87/1.23</td>
<td>0.29 ± 0.28</td>
</tr>
<tr>
<td>Year 9: bias correction</td>
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<tr>
<td>INR Range: i-STAT fingerstick vs Stago</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>0-7.2</td>
<td>103</td>
<td>0.92/1.18</td>
<td>0.16 ± 0.52</td>
</tr>
<tr>
<td>0-3.5</td>
<td>72</td>
<td>0.86/1.22</td>
<td>0.01 ± 0.32</td>
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Table 2
Changes in Long-Term i-STAT to Stago INR Bias

<table>
<thead>
<tr>
<th>Year</th>
<th>Average Bias ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.29 ± 0.40</td>
</tr>
<tr>
<td>2</td>
<td>0.20 ± 0.23</td>
</tr>
<tr>
<td>3</td>
<td>0.09 ± 0.23</td>
</tr>
<tr>
<td>4</td>
<td>0.22 ± 0.40</td>
</tr>
<tr>
<td>5</td>
<td>0.18 ± 0.25</td>
</tr>
<tr>
<td>6-8</td>
<td>0.73 ± 0.59</td>
</tr>
<tr>
<td>9</td>
<td>0.16 ± 0.52</td>
</tr>
</tbody>
</table>

* Bias for fingerstick capillary blood international normalized ratio (INR) tested on i-STAT was compared with venous plasma tested on Stago Compacts. Results shown are from initiation studies (year 1), combined annual 6-month correlation data (years 2-5), and from large comparison studies for years 6-8 and year 9.

observed during the first 5 years (0.16 INR units, slope 1.19; Figure 1D, Figure 2D, and Tables 1 and 2).

Bias between patient results from the i-STAT and Stago analyzers in the laboratory was determined at 6-month intervals to monitor correlation stability on an ongoing basis. Table 2 shows the average bias over time. The average bias remained below 0.3 INR over a 5-year period, unexpectedly increased in year 6, and returned to lower levels in year 9. During the 3-year period that showed increased bias, capillary blood i-STAT results became much higher without similar increases in the central laboratory Stago plasma results. To further evaluate the shift in bias seen after year 6, a method correlation was performed for 195 i-STAT vs Stago INR results (Figure 1C). In the early study, the slope had been 1.08, but in year 6 it increased to 1.48 (Figure 1C and Table 1), confirming the new onset of a larger proportional bias between the assays. In year 9, after recalibration and correction of the i-STAT method, the slope improved to 1.21 (Figure 1D). Representative Bland-Altman bias plots for years 1 to 5, years 6 to 8, and year 9 are presented in Figure 2, and related bias determinations are presented in Figure 2 and Table 1. Data from the first 5 years showed a significant agreement across a range of INR values for most patients tested with the Stago laboratory assay and the i-STAT method. In year 6, however, the limited bias between i-STAT fingerstick and Stago venous plasma data increased greatly from the initial 5-year average of 0.23 INR (Figure 2B) to 0.73 INR (Figure 2C). Following the calibration correction and drift adjustment in year 9, the bias improved to 0.16 INR, which was closer to that seen in years 1 to 5 (Figure 2D); the patient result correlation similarly improved even among many high INR levels (Figures 1B, 1C, and 1D).

During Early Studies High Bias Was Limited to a Subgroup of Patients With High INR Values

During the first 5 years, with assays demonstrating low bias and close correlation, there was an interest in understanding why a subset of patients in the high INR range had results with a higher bias. Fifty patients with INRs higher than 3.5 were separated into 2 groups based on whether i-STAT values were more than 0.5 INR above the Stago values. There were 29 patients (58%) with method differences at 0.5 INR or less, with a group correlation coefficient of 0.91 and group average bias of 0.1. There were 21 patients (42%) with differences greater than 0.5 INR. They were a population of patients with a correlation coefficient of 0.73 and a large average i-STAT bias that was 1.01 INR units higher than Stago (Table 1).
To identify patient characteristics associated with a highly increased i-STAT bias, charts of 8 patients were reviewed in whom multiple paired i-STAT and Stago results were collected because of persistently elevated i-STAT INR values. These data were collected over an extended testing period when the bias between the 2 assays was low and the correlation was stable. Correlation of i-STAT to Stago for the combined data from these 8 patients demonstrated a poor correlation coefficient of 0.81 compared with the total population correlation coefficient of 0.93 for the complete INR range (Figure 3A and Table 1). The average i-STAT bias for the group data was 1.36 INR units, a significant assay difference (Figure 2C and Table 1). One speculation is that certain clinical conditions contribute to a greater discrepancy for INR values when tested by i-STAT.

Interestingly, the increased slope for the data from these 8 patients (1.45) was similar to the increased slope that developed for all patients during the sixth year of the study (1.48) (Figure 1C, Figure 3A and Table 1). Further, after the correlation worsened in year 6, nearly all patients with an INR above 3.5 had i-STAT values that were increased more than 0.5 INR above the Stago values (Figure 1C).

**Patient Conditions Associated With INR Method Bias**

Potential reasons for increased i-STAT bias in the 8 patients with high i-STAT bias are presented in Table 3. Conditions associated with peripheral edema, such as congestive heart failure (CHF) and chronic obstructive pulmonary disease (COPD), as well as skin disease appeared in multiple patients. Two patients had inflammatory dermatitis, one of which was neutrophilic with papillary edema, which might differentially affect capillary results and not venous results. Other conditions possibly affecting INR determinations were also noted, including liver disease and coagulation abnormalities. Two INR discrepancy patterns were noted in this group of patients (Table 3). One group had elevated i-STAT values both within and above the therapeutic range, whereas INR values for the other group were higher only when the INR exceeded the therapeutic range (Table 3). Conditions associated with the first group were mitral valve disease or replacement, thrombosis, CHF, COPD, coagulation abnormalities, and inflammatory dermatitis. Conditions associated with the second group included aortic valve replacement, CHF, cardiomyopathy, and poor liver function with increased liver enzymes.

**Discussion**

At the study onset, an increased bias was considered probable because method-based disagreements among INR assays are common. The initial agreement was unexpected because i-STAT and Stago use different detection systems, different samples, and different thromboplastin sources calibrated to different IRPs that do not change in unison. The level of agreement for results above 3.5 INR units was also unexpected because INR values higher than 4.5 are typically not included in ISI calculations. The sixth-year transition from general agreement across a broad range of INR values to a notable bias for all high INR values appeared as
Thromboplastin Reagent Composition Affects Factor Sensitivity

Many possible causes for the increase in assay bias had been considered. Neither POC nor laboratory instrumentation was changed, and there were no notices of change in either reagent system. For instance, the synthetic substrate cleaved by thrombin in the i-STAT method remained the same. Therefore, the increased general bias seen in year 6 suggested development of a new reagent or calibration difference, despite stable ISI values. Stago rabbit thromboplastin and the i-STAT human recombinant thromboplastin are calibrated to separate WHO IRPs. Notably, the i-STAT cannot be directly calibrated because it uses capillary whole blood that needs to be referenced to a plasma method that is traceable to the WHO IRP. Stago thromboplastin reagent lots are changed annually with small changes in the ISI. The geometric mean is determined annually from grouped results collected across the network of hospitals and exhibits only small changes. Laboratories are quite aware of reagent lot changes but are generally not notified of changes in reagent formulation or lot standardizations a new event. The higher bias may have partly resulted from greater manifestation of inherent variables.

The loss of correlation was noted by and discussed among laboratories within and outside the VA system over several years, absent manufacturer acknowledgment, which was slow in developing. Differences in thromboplastin standardization or thromboplastin sensitivity to decreased factor levels seemed the most likely cause for the increase in i-STAT values. Such differences could also lead to greater assay bias at higher INR levels and account for greater differences associated with sample type and patient condition. Several inquiries suggested that correlation to the newest international reference plasma for recombinant tissue factor, which was released around the time of the bias onset, had not occurred. Eventually, in 2012, i-STAT thromboplastin standardization was corrected by correlation to a plasma assay traceable to the new international reference plasma rTF/09. The result was a standardization correction of 12% downward and a further downward adjustment by 8% to account for assay drift.

### Table 3

Two Clinical Data Patterns for 8 Individual Patients With High i-STAT Bias During a Period of Close Correlation

<table>
<thead>
<tr>
<th>Typical i-STAT INR Correlation Pattern</th>
<th>Cardiology</th>
</tr>
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<tbody>
<tr>
<td>Stago</td>
<td></td>
</tr>
<tr>
<td>i-STAT</td>
<td></td>
</tr>
<tr>
<td>Sex/Age, y</td>
<td></td>
</tr>
<tr>
<td>Indication for Warfarin</td>
<td></td>
</tr>
<tr>
<td>Heart Condition</td>
<td>CHF</td>
</tr>
<tr>
<td>CHF</td>
<td>Respiratory</td>
</tr>
<tr>
<td>CAD, coronary artery disease (may include myocardial infarction with bypass or stent placement); CHF, congestive heart disease; COPD, chronic obstructive pulmonary disease; INR, international normalized ratio.</td>
<td></td>
</tr>
</tbody>
</table>

#### a Study was performed during initial period of close and stable assay correlation. Data are included for the first patient in each category.
to the designated IRP. Long-term i-STAT product information showed the same ISI and geometric mean, yet there were clearly changes in patient INR levels in the patient population. Presumably, the relationship between thrombin generation and cleaving of the synthetic substrate to create an amperometric signal is consistent. There had been no evidence that either assay had been recalibrated to a new IRP preceding the shift in bias, and there is no available evidence that the i-STAT had been referenced to an IRP or the newer human recombinant IRP, rTF/09, until the increase in assay bias was corrected during year 9 of the study.

It is not clear why the i-STAT assay needed to be corrected by 8% for drift. In addition to referencing the assay to the IRP, unexpected assay discrepancies or drift could arise as a result of either changes in tissue or recombinant thromboplastin reagent sources or preparation changes that affect inherent sensitivities to factor levels. The observed shift in assay correlation was noticed in multiple VA hospital laboratories in our network, suggesting it did not correspond to a local change. It was previously observed that rabbit and human recombinant thromboplastin reagents exhibit INR increases at different levels of decreased factor activity. Factor sensitivity differences can account for different assay results seen in some patients with INR levels above 3.5. Notably, preparations of human recombinant thromboplastin reagents are known to vary in sensitivity to factor deficiencies with modifications in phospholipid composition and salt concentration, without changing the ISI values. Modifications in reagent preparation or source that do not affect the ISI could, therefore, contribute to a shift in assay bias and may account for the recent 8% assay correction for drift.

### Why Is Bias Greater for Select Individuals? Patient or Sample Differences Have Differential Effects on Thromboplastin Reagents

During periods of close correlation, a subset of patients still demonstrated greater bias between the assays. Characteristics of the assay reagents were compared and patient conditions reviewed to identify hypothetical causes for the unexplained bias.

Thromboplastin reagents differentially react to factor reduction and vitamin K inhibition by warfarin. Vitamin K antagonists decrease active forms of coagulation factors II, VII, IX, and X and generate partially carboxylated and decarboxylated forms of these factors, which are known as proteins induced by vitamin K absence/antagonism (PIVKA). These proteins differ in their synthetic rate and half-life and are therefore differentially affected by long-term warfarin therapy. The thromboplastin reagents used in the i-STAT and Stago assays would be expected to vary in sensitivity to decreased factor concentrations and in response to partially carboxylated factors, which can act as competitive inhibitors. Compared with human recombinant thromboplastin, rabbit thromboplastins are reported to be insensitive to the presence of PIVKA proteins. Such differences could account for some patient-specific differences in INR values between the 2 assays but are unlikely to account for the broader shift seen in year 6.

Liver disease adversely affects functional coagulation factor production by hepatocytes, and this is compounded by the presence of vitamin K antagonists. Liver function was impaired because of alcohol, hepatitis, or decreased liver perfusion in several study patients who had increased i-STAT INR values when above therapeutic range. The effects of chronic liver disease on INR levels are complex, and patients with liver impairment demonstrate poor INR correlations between the rabbit brain and human recombinant thromboplastins used in Stago and i-STAT assays. Use of the INR is not recommended in patients with liver disorders, which cause variations that may significantly influence the results.

Changes in hematocrit counts have been associated with variance in POC INR determinations using capillary whole

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<th>Dermatology</th>
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<td>Whole body inflammatory dermatitis</td>
<td>Enzyme increases</td>
<td>Factor V R506Q, 1 allele</td>
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<td>Neutrophilic dermatosis, edema, scleroderma</td>
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<td>Enzyme, bilirubin increases; hepatitis C; chronic ethanol</td>
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blood samples. High or low hematocrit counts were not the cause of assay bias in this study, however, because only 1 of the 8 patients studied for higher i-STAT bias had an abnormal (decreased) hematocrit count. Further, correlation data demonstrated only a moderately higher average INR bias for capillary fingerstick levels over venous whole blood levels on i-STAT. The assay bias appeared more likely to be associated with differences in coagulation factor concentrations.

Interestingly, 2 patients with inflammatory skin disease demonstrated i-STAT INR increases within and above the therapeutic range. Heart failure and obstructive lung disease were also common among patients with higher i-STAT INR values. A capillary specimen from edematous or inflamed tissue could cause an i-STAT result that is comparatively higher than the venous sample tested by the laboratory method.

Glucose levels are known to differ between venous and capillary samples and by anatomic site of the capillary sample collection. Accordingly, INR levels for capillary samples would likely be affected by status of the tissues around the capillary and capillary blood flow. Although difficult to test, it seems reasonable to assume that capillary edema or inflammation would contribute to dilution or altered capillary perfusion. Accordingly, patients with inflammatory skin disease or peripheral edema exhibited a notable bias for capillary samples.

One patient with inflammatory dermatitis tested positive for lupus anticoagulant. The use of some thromboplastin reagents are associated with prolonged PTs in the presence of lupus anticoagulant. This is not a feature of all thromboplastin reagents but may have relevance to the i-STAT assay because PT elongation by lupus anticoagulant has been associated with recombinant thromboplastins and with POC methods.

**Effect of Bias on Clinical Decision Making**

The clinical discrepancies created by the significant correlation change seen in this study are illustrated by the Shermock method for assessing agreement between the laboratory and POC INR methods. Figure 4 demonstrates the incidence of values falling outside the expected dose ranges. During the period of poor correlation (Figure 4A), 42% of i-STAT and Stago results did not agree, whereas after the correction of the i-STAT assay (Figure 4B), just 18% of results did not agree.

In conclusion, new onset of PT/INR assay disagreement may be best explained by thromboplastin reagent preparations with dissimilar sources, reference standards, formulation constancy, and sensitivities to factor level reductions. In this instance, the largest share of the difference seemed to be corrected by appropriately referencing the i-STAT recombinant thromboplastin to the most recent IRP; the remaining assay drift may relate to the other factors. Patient conditions that may decrease factor concentrations or affect capillary function may intensify the reagent differences. One means of resolving correlation differences between the POC and laboratory assay is to change one of the testing systems. As demonstrated here, however, current assay correlation may not predict future correlation. Data from previously published studies should...
also be used cautiously. In the VA healthcare system, and in other networks, detection of bias and trends may also be enhanced through monitoring larger repositories of laboratory data. Open interaction between manufacturers and clinical laboratories is required to accomplish timely recognition and correction of problems. It is necessary for the laboratory and the providers to continually evaluate the relationship between the assays, especially with limited information regarding changes in thromboplastin reagent formulation or standardization practices.

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References


